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Influence of foliar treatments on chlorophyll content in tomatoes (*Solanum lycopersicum* L.) under abiotic stress conditions

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Abstract
Foliar fertilization is a modern plant nutrition technique that ensures rapid nutrient absorption and supports photosynthetic processes. The aim of this study was to evaluate the influence of foliar fertilization on chlorophyll content in three tomato genotypes (Genotype 1 Buzau 1600, Pontica(Dacia) Florina 44 Genotype 2, Genotype 3) cultivated in southern Romania under abiotic stress conditions. The fertilization treatments were: V0 (control – without foliar fertilization), V1 (Albit + Atonik), and V2 (Albit + Atonik + Polyamin). ANOVA analysis showed that the plant phenological stage had a highly significant effect on chlorophyll content ($p < 0.001$), while the fertilization treatment and the phenological stage × fertilization interaction had no significant effect. Chlorophyll content ranged from 39 SPAD to 58 SPAD, with maximum values at phenological stage 5. The results suggest that the plant's developmental stage is the main factor influencing chlorophyll levels. Monitoring phenological stages is essential for optimal application of agronomic interventions, and the use of variants that maintain high chlorophyll levels in later stages (e.g., V2) can support photosynthetic efficiency and yield.

Keywords: tomatoes, foliar fertilization, chlorophyll, phenological stage, abiotic stress

Introduction

Tomatoes (*Solanum lycopersicum* L.) are among the most widely cultivated and consumed vegetables worldwide, due to their nutritional value and economic importance. They are an excellent source of vitamin C, potassium, folate, and bioactive compounds such as lycopene, a carotenoid known for its antioxidant activity and potential to reduce the risk of chronic diseases, including cardiovascular disorders and certain types of cancer [9]. In Romania, tomatoes hold a prominent place in horticultural crops, being extensively cultivated both for fresh consumption and processing [14,15].

Tomato production is particularly vulnerable to abiotic stresses, including drought, soil salinity, and extreme temperature fluctuations, all of which significantly affect plant growth, photosynthetic efficiency, and crop quality [2,13,16]. In Romania, the increasing frequency of drought episodes and climatic variability exacerbates these challenges, making the stabilization of tomato yields a priority [12].

Nutrient management, particularly fertilization, plays a key role in enhancing plant tolerance to abiotic stress. The balanced application of macro- and micronutrients — such as nitrogen, potassium, zinc, and magnesium — helps maintain photosynthetic activity, osmotic balance, and plant vigor under unfavorable conditions [4,7,11]. Organic fertilizers and biofertilizers are also recognized for their ability to improve soil structure, increase microbial activity, and enhance plant stress resistance [1].

Biostimulants are gaining increasing attention as a complementary tool to traditional fertilization strategies, improving plant metabolism and stress tolerance. For example, Atonik, a nitrophenolate-based growth stimulator, promotes root development, pollen germination, and fruit set [3]. Haifa Poly-Amin, containing amino acids and peptides, enhances metabolic activity and vegetative growth when applied foliarly [6]. Albit, a multifunctional biostimulant with antioxidant and elicitor properties, strengthens plant immunity and reduces oxidative damage under stress conditions [10]. However, the effectiveness of these products depends on several factors, including specific genotype characteristics, environmental conditions, and application protocols.

The present study aims to evaluate the influence of foliar fertilization on chlorophyll content in three tomato genotypes across different phenological stages under abiotic stress conditions. Chlorophyll is an essential physiological parameter, indicating photosynthetic capacity and plant health. Abiotic stress factors, such as drought and high temperatures, affect chlorophyll levels, limiting growth potential and yield. Foliar fertilization can complement nutrient supply by providing a rapid response at the leaf level, maintaining photosynthetic activity during critical periods [5,8].

Materials and Method

The study was conducted in 2024 in the southwestern region of Romania, in the commune of Vârvoru de Jos, Dolj County, an area characterized by a temperate-continental climate, with high susceptibility to drought and extreme temperatures.

Figure 1. The three genotypes (Florina, Buzau 1600 and Pontica Dacia)

The biological material consisted of three tomato genotypes (Solanum lycopersicum) widely cultivated in this region: Pontica (also known as Dacia), Buzău 1600, and Florina 44 (figure 1). These varieties were selected based on their agronomic importance, regional adaptation, and differing physiological responses to abiotic stress. Irrigation was controlled and applied via a drip system every 3 days, ensuring optimal and uniform soil moisture, thereby preventing severe water stress.

These experimental conditions allowed the evaluation of biostimulant treatments under controlled moisture levels, while plants were exposed to typical regional abiotic stress factors, such as temperature fluctuations and moderate climatic drought.

The foliar fertilization treatments applied were as follows:

V0: Atonik (10 mL/10 L water), applied once at the 10-leaf stage (May 20, 2024).

V1: Atonik (10 mL/10 L) at the 10-leaf stage + Albit (2 mL/10 L) applied three times (June 5, June 12, and July 12).

V2: Atonik (10 mL/10 L) at the 10-leaf stage + Albit (2 mL/10 L) applied three times + Poliamin applied foliarly three times (June 11 – 50 mL/10 L, June 26 – 100 mL/10 L, and July 7 – 100 mL/10 L).

All applications were performed early in the morning to reduce volatilization losses and maximize foliar absorption.

Measured parameters

An essential physiological parameter, chlorophyll content, was evaluated, reflecting the plants' photosynthetic capacity and overall health status. Chlorophyll was measured using a portable SPAD meter, which allows rapid and non-invasive estimation of leaf chlorophyll content. The data provided information on photosynthetic efficiency, the level of abiotic stress experienced by the plants, and their response to different foliar fertilization treatments. Measurements were performed on five randomly selected plants from each plot, and mean values were calculated for each plant replicate, allowing precise comparisons between phenological stages, genotypes, and fertilization treatments.

Growth intensity was measured by plant height (cm) using a ruler, recorded weekly throughout the vegetative period.

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Studied factors

Factor A – Phenological stage: a1 = 25.05.2024, a2 = 20.06.2024, a3 = 26.06.2024, a4 = 01.07.2024, a5 = 29.07.2024

Factor B – Foliar fertilization treatment: b1 = V0 (control), b2 = V1 (Albit + Atonik), b3 = V2 (Albit + Atonik + Poliamin)

The experiment was arranged as a two-factor factorial design, with 4 replications for each phenological stage × treatment combination.

Chlorophyll determination

Chlorophyll content was evaluated using a SPAD meter at five vegetation phenological stages (figure 2).

Statistical Analysis

Figure 2. Chlorophyll content evaluation

Post-ANOVA tests (LS and Duncan) were applied to identify differences between means, allowing the separation of significantly different means at p < 0.05 and p < 0.01 levels.

Results and Discussion

The data were analyzed using a two-way ANOVA, and differences between means were tested using the Student's t-test for phenological stages and Duncan's test for interactions.

The general structure of the analysis of variance is presented in Table 1, showing the sources of variation, degrees of freedom (DF), sums of squares (SS), mean squares (MS), and the corresponding F-ratios.

1. Analysis of Variance (ANOVA) — Effects of Phenological Stage and Treatment on Chlorophyll Content

Table 1. Two-way ANOVA (phenological stage × treatment × replications) for chlorophyll content (SPAD)

Source of Variation	Degrees of Freedom [DF]	Sum of Squares [SS]	Mean Square [MS]	F-statistic
Factor A (Phenological Stage)	a − 1	SSA	MSA = SSA / DFA	FA = MSA / MSerror
Factor B (Treatment)	b − 1	SSB	MSB = SSB / DFB	FB = MSB / MSerror
AB (Interaction)	(a − 1)(b − 1)	SSAB	MSAB = SSAB / DFAB	FAB = MSAB / MSerror
Error	a × b × (r − 1)	SSerror	MSerror = SSerror / DFerror	–

Legend: ns — not significant; *** — p ≤ 0.001

The analysis of variance revealed that the phenological stage had a statistically significant effect (p < 0.01) on chlorophyll content expressed as SPAD values. This confirms the variation of chlorophyll content according to the developmental stage of the plants, correlated with the dynamics of physiological processes and the degree of leaf maturation.

Moreover, Factor B (treatment variant) caused significant differences (p < 0.05) between treatments, indicating the influence of applied technological conditions (fertilization, moisture regime, foliar treatments, etc.) on chlorophyll accumulation. The A × B interaction effect was also significant, suggesting that the plant response to applied treatments depends on the analyzed growth stage. Consequently, the impact of experimental variants is not uniform throughout the growth cycle but varies according to phenological stage.

The data in Table 1.2 present the mean SPAD values (±SE) for the phenological stage factor (A) — representing different plant developmental stages — providing a clear picture of chlorophyll dynamics in the leaves during the vegetative period.

The mean SPAD values shown in Table 1 highlight a significant variation in chlorophyll content depending on the phenological stage. The SPAD index, which indirectly reflects leaf chlorophyll concentration

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and, consequently, photosynthetic activity, exhibited a dynamic evolution throughout the studied period. At the first measurement (a1 — 25.05.2024), a low mean SPAD value (39.1 ± 1.438) was recorded, characteristic of early vegetative stages, when leaves are still developing and the accumulation of photosynthetic pigments is limited.

In the subsequent phenological stages (a2 — 20.06.2024 and a3 — 26.06.2024), the mean SPAD values increased significantly, reaching a maximum at a3 (54.5 ± 0.957). This increase reflects leaf maturation and the intensification of photosynthetic processes, characteristic of the period of maximum physiological activity of the plants.

After this peak, at stage a4 (01.07.2024), a slight decrease in mean SPAD value (50.2 ± 1.517) was observed, possibly due to the onset of leaf senescence and the redistribution of nutrients (especially nitrogen) to reproductive organs. At the final analyzed phenological stage (a5 — 29.07.2024), the mean SPAD value increased again (58.4 ± 2.136), but with greater variability according to the confidence interval (53.674–63.076). This may indicate differences between young and older leaves or an adaptive response of the plants to environmental conditions during this period.

The overall SPAD index trend demonstrates a clear correlation between developmental stage and chlorophyll content, with maximum values recorded during the physiological maturity of leaves. The results suggest that phenological stage is a key factor determining the variation in chlorophyll content, directly influencing the plants' photosynthetic potential and nutrient use efficiency.

Means and Standard Errors — Effect of Phenological Stage (A), Treatment Variant (B), and A × B Interaction

The soil in the experimental area was classified as cambic chernozem, with moderate fertility and good drainage capacity. Prior to the experiment, soil samples were collected from the experimental plots to determine their physico-chemical characteristics and assess suitability for tomato cultivation. The results are summarized in Table 2.

Table 2. Physico-chemical characteristics

Sample No.	pH (H_2O, 1:2.5)	Organic Matter (%)	Total Nitrogen (%)	Available Phosphorus (ppm)	Available Potassium (ppm)
1	6.40	2.529	0.220	408.0	696
2	7.88	2.738	0.238	295.0	730
3	7.81	2.552	0.222	276.2	557

Soil pH values ranged between 6.40 and 7.88, with most values within the optimal range for tomato cultivation (6.0–7.5). Organic matter content varied between 2.529 and 2.738%, slightly below the optimal humus content (3.5–6.6%). Total nitrogen content was slightly below the optimal range of 0.25–0.35%, indicating moderate fertility. Available phosphorus levels ranged from 276.2 to 408 ppm, generally above the optimal range (161–361 ppm), while available potassium ranged between 557 and 730 ppm, within the optimal range (374–911 ppm). Overall, soil conditions are suitable for tomato cultivation, although careful nutrient management is recommended.

Table 3. SPAD values for the phenological stages.

Phenological Stage	Mean SPAD	Std. Err.	95% CI Lower	95% CI Upper
a1 — 25.05.2024	39.1	1.438	35.968	42.298
a2 — 20.06.2024	52.0	1.000	49.799	54.201
a3 — 26.06.2024	54.5	0.957	52.393	56.607
a4 — 01.07.2024	50.2	1.517	46.828	53.505
a5 — 29.07.2024	58.4	2.136	53.674	63.076

The mean SPAD values ranged from 39.1 (phenological stage 1) to 58.4 (phenological stage 5). Differences among the phenological stages were confirmed as statistically significant by the F-test ($p < 0.001$).

Effect of the Experimental Variant (Factor B) on Chlorophyll Content (SPAD)

The mean SPAD values for the treatment variant factor (B) are presented in Table 3. It can be observed that the differences between variants are relatively small, suggesting a moderate effect of the applied treatments on leaf chlorophyll content.

Table 4. SPAD values for the fertilization treatments.

Variant	Mean SPAD	Std. Err.	95% CI Lower	95% CI Upper
V0	51.3	1.878	47.408	55.272
V1	50.3	1.504	47.102	53.398
V2	50.9	2.132	46.452	55.378

The analysis of mean values shows that the control variant (V0) recorded the highest SPAD index value (51.3 ± 1.878), closely followed by V2 (50.9 ± 2.132) and V1 (50.3 ± 1.504). The differences between means are small, and the 95% confidence intervals overlap considerably, indicating that the variations are not statistically significant.

This result suggests that the treatments applied in the experimental variants did not produce significant changes in leaf chlorophyll content compared to the control. Moreover, the similar standard errors (±1.50–2.13) confirm good uniformity of the experimental data, without major deviations between replications. From a physiological perspective, the constant SPAD values can be explained by a balanced state of nutrition and photosynthetic processes in the plants. It can be assumed that, under the experimental conditions of the study year, environmental factors (light, temperature, humidity) and the general level of fertilization were favorable, and the applied treatments did not induce sufficient variation to significantly alter chlorophyll accumulation.

The mean SPAD values for the combinations of phenological stage (A) and treatment variant (B) are presented in Table 5. Analysis of these data allows us to observe how the applied treatments influenced chlorophyll content at different stages of plant development.

Table 5. SPAD values for phenological stage x fertilization treatment interactions.

Phenological Stage	**Variant**	**Mean SPAD**	**Std. Err.**	**95% CI Lower**	**95% CI Upper**
a1 — 25.05.2024	b1 – V0	37.6	0.978	34.439	40.661
	b2 – V1	43.0	3.358	32.289	53.661
	b3 – V2	36.9	1.749	31.310	42.440
a2 — 20.06.2024	b1 – V0	52.0	0.577	50.163	53.837
	b2 – V1	52.3	2.658	43.793	60.707
	b3 – V2	51.8	1.887	45.743	57.757
a3 — 26.06.2024	b1 – V0	57.8	0.854	55.032	60.468
	b2 – V1	54.0	1.291	49.891	58.109
	b3 – V2	51.8	1.250	47.772	55.728
a4 — 01.07.2024	b1 – V0	52.0	1.080	48.563	55.437
	b2 – V1	47.8	3.838	35.536	59.964
	b3 – V2	50.8	2.496	42.807	58.693
a5 — 29.07.2024	b1 – V0	57.4	4.352	43.549	71.251
	b2 – V1	54.3	2.395	46.652	61.898
	b3 – V2	63.5	3.317	52.893	74.007

The interaction between phenological stage and treatment variant shows a distinct evolution of chlorophyll content depending on the developmental stage and applied treatment. In the first phenological stage (a1 — 25.05.2024), SPAD values were low across all variants, with a slight increase in V1 (43.0) compared to the control (37.6). However, these differences fall within the standard error limits, suggesting that at the beginning of the vegetative period, the treatments did not produce consistent effects on chlorophyll synthesis.

In the second phenological stage (a2 — 20.06.2024), SPAD values increased significantly in all variants (≈52), indicating pronounced chlorophyll accumulation with leaf development. Differences between variants were minimal, showing a uniform response of the plants to the treatments.

During the third phenological stage (a3 — 26.06.2024), maximum values were observed for the control variant (57.8), followed by V1 (54.0) and V2 (51.8). This peak coincides with the period of maximum photosynthetic activity, and the higher values in the control suggest that the treatments did not further stimulate chlorophyll accumulation, possibly due to an already optimal level.

In the fourth phenological stage (a4 — 01.07.2024), SPAD values began to decrease, indicating the onset of leaf senescence. Variant V1 recorded the lowest value (47.8), while V0 and V2 maintained higher chlorophyll content (52.0 and 50.8, respectively).

In the final phenological stage (a5 — 29.07.2024), a divergent trend was observed: variant V2 recorded the highest SPAD value (63.5), surpassing the control (57.4) and V1 (54.3). This difference suggests a positive interaction between V2 treatment and the late phenological stage, indicating a potential stimulatory effect on chlorophyll synthesis or a delay in leaf senescence.

The SPAD index evolution depending on the phenological stage × variant combination shows that plant responses to treatments are not constant throughout the vegetative period. In the early stages, chlorophyll content is predominantly determined by the physiological development of the leaves, with minimal influence from the treatments. In contrast, in the later stages, certain variants (especially V2) may contribute to maintaining photosynthetic activity for a longer period, reflected by higher SPAD values.

To highlight the significance of differences between phenological stages in chlorophyll content, expressed as the SPAD index, the Student's t-test relative to the overall mean of the experiment was applied. The results are presented in Table 6.

Table 6. Comparison of SPAD means for phenological stage.

Phenological Stage	Chlorophyll [SPAD]	% of Mean	Difference [SPAD]	Significance
a1 — 25.05.2024	39.1	77.0	-11.7	000
a2 — 20.06.2024	52.0	102.3	1.2	ns
a3 — 26.06.2024	54.5	107.2	3.7	ns
a4 — 01.07.2024	50.2	98.7	-0.7	ns
a5 — 29.07.2024	58.4	114.8	7.5	***
Overall Mean	50.8	100.0	—	mt

LSD: 5% = 3.99 SPAD; 1% = 5.32 SPAD; 0.1% = 6.97 SPAD

The results of the Student's t-test show that differences between phenological stages vary in significance, reflecting a clear dynamic of chlorophyll content throughout the vegetative period. In the first phenological stage (a1 — 25.05.2024), the mean SPAD value (39.1) was significantly lower than the overall mean (50.8), with a difference of −11.7 SPAD being highly significant ($p < 0.001$). This confirms that during early stages, leaves contain low amounts of chlorophyll because pigment formation is not yet complete.

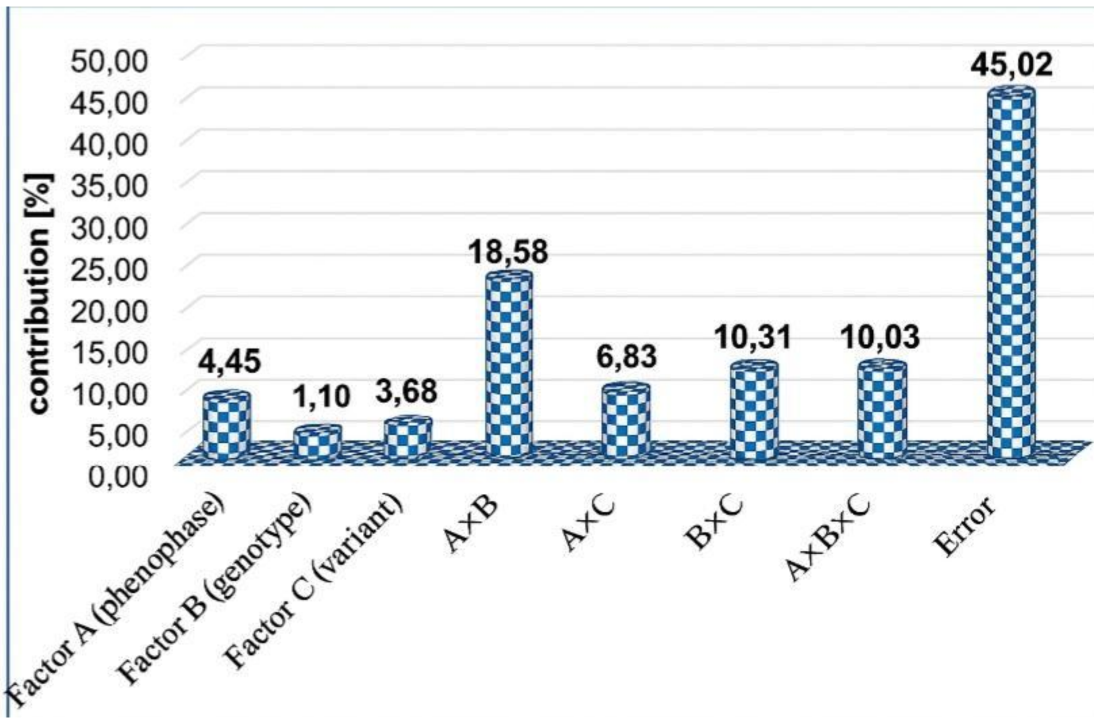

Figure 3. Contribution of factors A[phenophase], B[genotype], C[variant] and interactions

In the second (a2) and third (a3) phenological stages, SPAD values (52.0 and 54.5) slightly exceeded the overall mean, but the differences were not statistically significant (ns). These stages correspond to the physiological maturity of the leaves, when chlorophyll content reaches a stable maximum, and the observed variations are natural and insignificant.

In the fourth phenological stage (a4 — 01.07.2024), the SPAD value (50.2) was close to the overall mean, with a difference of −0.7, which is not significant. This suggests stabilization of chlorophyll content during this period.

In the final phenological stage (a5 — 29.07.2024), the highest SPAD value was recorded (58.4), with a positive difference of +7.5 SPAD compared to the overall mean, which was highly significant ($p < 0.001$). This result indicates a pronounced increase in chlorophyll content, possibly associated with leaf renewal or the prolonged photosynthetic activity of newly formed leaves.

The evolution of SPAD values across phenological stages highlights a close correlation between leaf development and chlorophyll accumulation.

- In the early stages, the low chlorophyll content is determined by the incomplete formation of mesophyll tissues and limited photosynthetic activity.
- In the intermediate stages, high and stable values reflect the physiological maximum of chlorophyll, essential for sustaining intense photosynthesis.
- In the late stages, increases in SPAD values at certain points (a5) can be explained by compensatory processes or the emergence of new leaves, which exhibit higher chlorophyll concentration per unit area.

Factor A[phenophase] contributes to the production of chlorophyll in a proportion of 4.5%, factor B[genotype] contributes with 1.1%, factor C[variant] contributes with 3.7%. Among the interactions: AxB contributed with 18.6%, followed by BxC [10.3%], AxBxC 10%] and AxC [6.8%].
In conclusion, the greatest contribution has the interaction AxB followed by factors BxC and AxBxC.

Conclusions

The analysis of chlorophyll content, expressed by the SPAD index, revealed significant variation primarily determined by the phenological stage, which exerted the greatest influence on the level of green pigment. Mean SPAD values increased progressively from the beginning of the vegetative period (39.1 SPAD) to reach the physiological maximum (54.5 SPAD), and a further significant increase was recorded in the final phenological stage (58.4 SPAD; $p < 0.001$), indicating possible photosynthetic reactivation or late leaf regeneration.

Differences among the experimental variants (T0, T1, T2) were small and statistically non-significant, suggesting that the applied treatments did not substantially influence chlorophyll synthesis, with pigment content being mainly determined by the developmental stage and physiological condition of the plants.

However, the phenological stage × fertilization interaction (A × B) showed a differentiated physiological response, with high SPAD values during the peak photosynthetic stages (a2–a3) and a clear positive effect of variant V2 in the late stage (a5), indicating prolonged maintenance of photosynthetic activity.

Overall, the results confirm that the SPAD index is a reliable indicator of the physiological status of leaves, sensitive to phenological variations and capable of reflecting changes in chlorophyll metabolism. Its dynamics highlight the succession of leaf formation, maturation, and senescence processes, providing a solid basis for evaluating plant physiological efficiency and optimizing crop management practices.

Monitoring phenological stages is essential for the optimal timing of agronomic interventions, and the use of variants that maintain high chlorophyll levels in the late stages (e.g., V2) can support photosynthetic efficiency and yield. The SPAD index is recommended as a rapid, non-invasive method for assessing leaf physiological status, and multi-year studies correlated with yield are recommended to optimize cultivation strategies.

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